DATA EVALUATION RECORD
LIFE-CYCLE SEDIMENT *Chironomus tentans* TOXICITY TEST
(FOLLOWING EPA TEST METHOD 100.5)

1. **CHEMICAL:** Permethrin PC Code No.: 109701

2. **TEST MATERIAL:** Permethrin Purity: 97.7%
3. **CITATION:**

Authors: Picard, C.R.
Title: Permethrin – Life-Cycle Toxicity Test Exposing Midges (*Chironomus dilutus*) to a Test Substance Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods.
Study Completion Date: March 29, 2012
Laboratory: Smithers Viscient
790 Main Street
Wareham, MA 02571-1037
Sponsor: Consumer Specialty Products Assoc for the Permethrin Data Group II Steering Committee/Joint Venture
1667 K St, NW, Suite 300
Washington, DC 20006
Laboratory Report ID: 13981.6104
MRID No.: 48788101
DP Barcode: 401668

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: Christie E. Padova **Date:** 11/23/12

APPROVED BY: Teri S. Myers, Senior Scientist, CDM Smith

Signature: Teri S Myers **Date:** 10/11/14

5. **APPROVED BY:**

Signature: **Date:**

6. **STUDY PARAMETERS**

Scientific Name of Test Organism:	*Chironomus dilutus*
Age of Test Organism:	2 hours old
Definitive Test Duration:	63 days
Study Method:	Intermittent flow-through
Type of Concentrations:	Mean-measured sediment concentrations, bulk and OC-normalized

7. CONCLUSIONS:

Results Synopsis:

BASED ON BULK SEDIMENT

Day 20 Survival:
NOAEC: 81 µg ai/kg
LOAEC: 137 µg ai/kg

Day 20 Growth (AFDW):
NOAEC: 137 µg ai/kg
LOAEC: >137 µg ai/kg

Percent Emergence:
NOAEC: 10 µg ai/kg
LOAEC: 22 µg ai/kg

Male Development Rate:
NOAEC: 81 µg ai/kg
LOAEC: 137 µg ai/kg

Female Development Rate:
NOAEC: 81 µg ai/kg
LOAEC: 137 µg ai/kg

Combined Male and Female Development Rate:
NOAEC: 49 µg ai/kg
LOAEC: 81 µg ai/kg

Combined Male and Female Days to Death:
NOAEC: 22 µg ai/kg
LOAEC: 49 µg ai/kg

Number of eggs per mated female
NOAEC: 137 µg ai/kg
LOAEC: >137 µg ai/kg

Number of Eggs per Primary Egg Case
NOAEC: 81 µg ai/kg
LOAEC: >81 µg ai/kg

BASED ON OC-NORMALIZED SEDIMENT

Day 20 Survival:
NOAEC: 4300 µg ai/kg TOC
LOAEC: 7200 µg ai/kg TOC

Day 20 Growth (AFDW):
NOAEC: 7200 µg ai/kg TOC
LOAEC: >7200 µg ai/kg TOC

Percent Emergence:
NOAEC: 520 µg ai/kg TOC
LOAEC: 1200 µg ai/kg TOC

Male Development Rate:
NOAEC: 4300 µg ai/kg TOC
LOAEC: 7200 µg ai/kg TOC

Female Development Rate:
NOAEC: 4300 µg ai/kg TOC
LOAEC: 7200 µg ai/kg TOC

Combined Male and Female Development Rate:
NOAEC: 2600 µg ai/kg TOC
LOAEC: 4300 µg ai/kg TOC

Combined Male and Female Days to Death:
NOAEC: 1200 µg ai/kg TOC
LOAEC: 2600 µg ai/kg TOC

Number of eggs per mated female
NOAEC: 7200 µg ai/kg TOC
LOAEC: >7200 µg ai/kg TOC

Number of Eggs per Primary Egg Case
NOAEC: 4300 µg ai/kg TOC
LOAEC: >4300 µg ai/kg TOC

BASED ON POREWATER

Day 20 Survival:

NOAEC: 0.757 µg ai/L
LOAEC: 1.17 µg ai/L

Day 20 Growth (AFDW):
NOAEC: 1.17 µg ai/L
LOAEC: >1.17 µg ai/L

Percent Emergence:
NOAEC: 0.390 µg ai/L
LOAEC: 0.234 µg ai/L

Male Development Rate:
NOAEC: 0.757 µg ai/L
LOAEC: 1.17 µg ai/L

Female Development Rate:
NOAEC: 0.757 µg ai/L
LOAEC: 1.17 µg ai/L

Combined Male and Female Development Rate:
NOAEC: 0.439 µg ai/L
LOAEC: 0.757 µg ai/L

Combined Male and Female Days to Death:
NOAEC: 0.234 µg ai/L
LOAEC: 0.439 µg ai/L

Number of eggs per mated female
NOAEC: 1.17 µg ai/L
LOAEC: >1.17 µg ai/L

Number of Eggs per Primary Egg Case
NOAEC: 0.0757 µg ai/L
LOAEC: >0.0757 µg ai/L

Endpoints affected: Day-20 survival, percent emergence, male and female (separate and combined) development rate, and combined male and female days to death
Most sensitive endpoint(s): percent emergence

8. ADEQUACY OF THE STUDY:

A. Classification: Acceptable/Supplemental/Unacceptable

B. Rationale: This study followed methods described in the "Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates", 2nd Edition, Test Method 100.5: "Life-cycle Test for Measuring the Effects of Sediment-associated Contaminants on *Chrionomus tentans*." March 2000 (EPA 600/R-99/064).

C. Reparability: N/A

9. MAJOR GUIDELINE DEVIATIONS:

This study does not fulfill any current U.S. EPA guideline requirement. Deviations from U.S. EPA Test Method 100.5 included:

1. The mean number of eggs per egg mass was 798 for the negative control level, which was just below the minimum required for study validity of ≥800 eggs/mass.

10. SUBMISSION PURPOSE: RED

11. MATERIALS AND METHODS

A. Test Organisms/Acclimation

Guideline Criteria	Reported Information
Species *Chironomus dilutus* (formerly *C. tentans*)	*Chironomus dilutus*
Life Stage <24-hr old larvae	2-hr old larvae
Source Collect a min. of 6 to 8 egg cases.	Egg masses were obtained from the laboratory's culture facility.
All organisms from the same source?	Yes

Guideline Criteria	Reported Information
Culture Conditions Transfer all egg cases to a crystallizing dish containing control water. Discard larvae that have already left the cases. Test organisms must be cultured and tested at 23°C, ideally in the same water used for testing.	Egg masses were placed in 270-mL crystallizing dishes containing approximately 200 mL of laboratory well water. The egg masses were reared at 23°C, observed daily until hatch, and then transferred into clean laboratory water to encourage the larvae to leave the egg mass.
Feeding	Midge larvae were fed daily a finely-ground flaked fish food suspension (4.0 mg/mL).

B. Test System

Guideline Criteria	Reported Information
Dilution water (overlying water) Culture water, well water, surface water, site water, or reconstituted water	Laboratory well water characterized as having a total hardness and total alkalinity of 56 to 74 and 20 to 29 mg/L as $CaCO_3$, respectively, a pH range of 6.9 to 7.7, and a specific conductivity range of 270 to 440 µmhos/cm.
Does water support test animals without observable signs of stress?	Yes
Water Temperature 23 ± 1°C	Daily measurements: 22 to 24°C Continuous measurements: 21 to 24°C
pH	6.2 to 7.4
Dissolved Oxygen >2.5 mg/L	3.5 to 8.7 mg/L
Ammonia Should not vary more than 50%.	≤0.10 to 0.60 mg/L as N
Hardness Should not vary more than 50%.	52 to 68 mg/L as $CaCO_3$

Guideline Criteria	Reported Information
Alkalinity Should not vary more than 50%.	20 to 30 mg/L as $CaCO_3$
Conductivity	330 to 390 µmhos/cm
Test Sediment Natural or formulated sediment	Artificial sediment (Batch No. 030811) was prepared according to OECD Guideline No. 218 (2004) by mixing the following components (on a dry weight basis): 2.4 kg sphagnum peat, 8.0 kg kaolin clay, and 29.6 kg fine sand (6.0, 20, and 74%, respectively). While blending using a large-scale mixer, 8 L of laboratory well water was also added. Prior to use, the sphagnum peat was pre-soaked in dilution water for 1 week. During this time, the peat was amended with 80 g of powdered $CaCO_3$ to increase the pH from 3.3 to 5.6.
Sediment Characterization pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content should be reported	Particle distribution – 81% sand, 4% silt, 15% clay (sandy loam) TOC – 1.9% Percent solids – 67.16% pH – 6.8 Ammonia concentration of pore water on Day 0 in the control group – 4.5 mg/L (as N)

Guideline Criteria	Reported Information
Test Material	Permethrin IUPAC name: 3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate CAS name: (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate Description: not reported Batch no.: PMPS000014 Purity: 97.7% (w:w) Storage: room temperature in the original container in a dark, ventilated cabinet A 60 µg ai/L primary stock solution was prepared by bringing *ca.* 0.00615 g of test substance (0.00601 g ai) to 100 mL with acetone. The stock was clear and colorless with no visible undissolved test substance. Five individual dosing stock solutions were prepared by diluting the appropriate volume (1.48 to 22.8 mL) of primary stock into 25 mL acetone.
Solvents	Acetone, 10 mL/2.7364 kg dw The acetone was allowed to completely evaporate during the mixing procedure. Both solvent control and negative control groups were included in the study.

Guideline Criteria	Reported Information
Sediment Spiking	A jar-rolling technique was used to apply the test substance to the sediment. A 10-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.050 kg of fine silica sand and the solvent was allowed to evaporate off for 45 minutes. The dry sand was then added to 4.0 kg of wet sediment (total of 2.7364 kg dw) in individual glass jars. Each jar was then rolled for 4 hours at *ca*. 15 rpm. The range of nominal concentrations (13 to 200 µg ai/kg dw) was based upon the results of a preliminary range-finding study and in consultation with the Sponsor.
Sediment Conditioning	The jars containing treated sediment were stored upright at 2 to 8°C for a 14-day equilibration period. Once a week during the equilibration period and prior to being added into the replicate exposure vessels, the jars were mixed on the rolling mill for 2 hours to ensure the sediment was homogeneous.

Guideline Criteria	Reported Information
Test Vessels 300 mL high-form lipless glass beakers containing 100 mL of sediment and 175 ml of overlying water	300-mL glass vessels, each with two slots cut on the top edge covered with 40-mesh Nitex® screen for drainage. Each vessel contained 100 mL (*ca.* 4.0-cm layer) of sediment (equivalent to 107 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at approx. 275 mL. On Day 17, emergence traps were placed over the test vessels to trap emergent flies for the remainder of the test. The emergence traps were 3.5-cm tall Plexiglass® tubes (id 6 cm) covered with wide-mesh Nitex® screen.
Reproductive/oviposit chambers	Plexiglass tubes (3.5-cm length, i.d. 6 cm) covered on the top with wide-mesh Nitex® screen and placed within a 100 x 20-mm Petri dish. With midge addition, 50 mL of laboratory well water was added to the chambers.
Type of Dilution System Continuous or intermittent	Intermittent flow-through
Flow Rate 2 volume additions/day	Days -1 to 2: 2 volume additions/day Days 2 to 63: 4 volume additions/day Flow-rate was increased due to low dissolved oxygen levels.
Aeration None, unless DO in overlying water drops <2.5 mg/L	None reported

Guideline Criteria	Reported Information
Photoperiod 16 hours light, 8 hours dark using wide-spectrum fluorescent lights; intensity of 100 to 1000 lux	16-hour light/8-hour dark photoperiod using fluorescent bulbs at an intensity range of 290 to 620 lux.
Feeding	Midge larvae were fed daily a finely-ground flaked fish food suspension (4.0 mg/mL). During exposure, the food was introduced at a rate of 1.5 mL of flaked fish food suspension per test vessel, once daily.

C. Test Design

Guideline Criteria	Reported Information
Duration About 50 to 65 days; each treatment is ended separately when no additional emergence occurs for 7 consecutive days. When no emergence is recorded from a treatment, termination of that level is based on the control sediment using the 7-day criterion.	63 days
Sediment Into Test Chambers One day prior (Day -1) to start of test: each sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	Test systems were established on Day -1. Overlying water was gently added using a turbulence reducer, and each vessel was placed under the renewal system. Additional test systems were established on Day 9 for auxiliary male production.

Guideline Criteria	Reported Information
Renewal of Overlying Water Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.	The overlying water was renewed via an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning.
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Test systems were observed daily for dead organisms (larvae or pupae) on the sediment surface, organism behavior (e.g., sublethal effects), and characteristics of test solutions. Reproductive/oviposit chambers were checked daily for dead adults and egg masses and dead flies were removed daily.

Guideline Criteria	Reported Information
Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment.	Preliminary toxicity assessment • 44-day exposure at nominal levels of 0 (negative and solvent controls), 0.025, 0.25, 2.5, 25, and 250 µg ai/kg • Twelve, <24-hour old larvae per replicate, with eight replicates per level established on Day 0 plus two replicates per level established on Day 10 (auxiliary male production) • Day-20 survival averaged 89, 89, 81, 72, 72, 78, and 28%, respectively (significant at the 250 µg ai/kg level; this level excluded from further statistical analyses) • No other treatment-related statistical differences were observed up to and including the 25 µg ai/kg level for the remaining parameters, i.e., Day-20 AFDW, percent emergence, male or female emergence rate, male or female days to death, egg masses per mated female, eggs per egg mass, number of eggs per mated female, percent hatch, or days to oviposition
Nominal Concentrations of Definitive Test	0 (negative and solvent controls), 13, 25, 50, 100, and 200 µg ai/kg sediment
Number of Test Organisms 12 organisms per test chamber are recommended; 16 replicates per treatment should be used (twelve at Day -1 and four for auxiliary males on Day 10)	12 organisms per test chamber 16 biological replicates per level
Test organisms randomly or impartially assigned to test vessels?	Yes

Guideline Criteria	Reported Information
Water Parameter Measurements Conductivity, hardness, alkalinity, and ammonia should be measured in all treatments at the beginning, on Day 20, and at the end of the test. Conductivity should also be determined weekly. DO and pH should be measured at the beginning of the test and at least three times per week thereafter. Temperature should be measured daily in one test chamber from each treatment. The temperature of the water bath should be continuously monitored.	For all levels, total hardness, alkalinity, specific conductance, and ammonia concentrations were measured in a composite sample of the overlying water on Days 0, 10, 20, and 63. DO, temperature, and pH were measured in the overlying water of each replicate vessel on Days 0, 10, 20, and 63. On the remaining days, DO and temperature were measured in one alternating replicate from each level, and the temperature was continuously monitored in an auxiliary vessel in the water bath.
Chemical Analysis	Concentrations of permethrin were determined in sediment and pore water of surrogate test vessels on Days 0, 20, and 63 using GC/MS. Following removal of the overlying water, the sediment was centrifuged at *ca*. 10,000 *g* for 30 minutes. The pore water was removed, and sediment (mixed well) and aqueous samples were extracted and analyzed using GC/MS based on methodology validated at Springborn Smithers (see Reviewer's Comments section for further details).

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in accordance with GLP Standards as specified in 40 CFR 160 with the following exceptions: routine water and food contaminant screening analyses for pesticides, PCBs, and toxic metals. These analyses, however, were performed using certified laboratories and standard validated methods.
Control Criteria **Was control survival at least 70% and dry weight at least 0.6 mg/organism (0.48 mg/organisms AFDW) on Day 20?** **Was control emergence ≥50%?** **Was the mean number of control eggs/egg case ≥800 and was the percent hatch ≥80%?**	All criteria met. Negative control: 83% and 1.45 mg/larva Solvent control: 88% and 1.11 mg/larva Negative control: 77% Solvent control: 60% Negative control: 798 eggs/case and 97% Solvent control: 865 eggs/case and 99%
Percent Recovery of Chemical	Procedural recoveries conducted concurrently with sample analysis: Sediment: Recovery range of 78.7 to 94.7% LOQ $_{sediment}$ = 0.98 to 2.1 µg ai/kg Aqueous: Recovery range of 92.4 to 111% (excludes one outlier of 250% on Day 63 at the 0.0300 µg ai/kg fortification level) LOQ $_{water}$ = 0.039 to 0.073 µg ai/L

Guideline Criteria	Reported Information
Data Endpoints	Day 20: - Survival - Ash-free dry weight (AFDW) Day 63: - Percent emergence - Male and female emergence rates - Male and female days to death (for mated midges) - Eggs masses per mated female - Eggs per egg mass - Eggs per mated female - Percent hatch - Days to oviposition
Raw data included?	Yes, sufficient.

Effects Data

20-Day Endpoints:

Following 20 days, survival averaged 83 and 88% for the negative and solvent control levels, respectively, compared to 92, 83, 67, 75, and 6% for the mean-measured (up to Day 20) 11, 25, 52, 92, and 180 µg ai/kg treatment levels, respectively. The difference was statistically-significant compared to the negative control at the 180 µg ai/kg level ($p<0.05$), and this level was subsequently excluded from further statistical comparisons. Using mean-measured concentrations, the NOAEC and LOAEC for 20-Day survival were 92 and 180 µg ai/kg, respectively, and the 20-Day LC_{50} (with 95% C.I.) was 110 (95 to 120) µg ai/kg. Adjusted for the organic carbon content of the sediment (i.e., 1.9%), the NOAEC and LOAEC for 20-Day survival were 4.8 and 9.5 µg ai/g TOC, respectively, and the LC_{50} was 5.7 (5.0 to 6.5) µg ai/g TOC.

Mean ash-free dry weights (AFDW) on Day 20 were 1.45, 1.11, 1.22, 1.30, 1.78, 1.12, and 0.76 mg per larva for the negative control, solvent control, and mean-measured (up to Day 20) 11, 25, 52, 92, and 180 µg ai/kg treatment levels, respectively. No statistically-significant differences were indicated up to and including 92 µg ai/kg. The 180-µg ai/kg level was excluded due to a significant effect on survival at this level. Using mean-measured concentrations, the NOAEC and LOAEC for 20-Day AFDW were 92 and >92 µg ai/kg, respectively, and the 20-Day EC_{50} was observed to be >180 µg ai/kg. Adjusted for the organic carbon content of the sediment (i.e., 1.9%), the NOAEC and LOAEC for

20-Day AFDW were 4.8 and >4.8 µg ai/g TOC, respectively, and the observed EC_{50} was >9.5 µg ai/g TOC.

Nominal Sediment, µg ai/kg	Mean-measured (Days 0 and 20 only) Sediment, µg ai/kg [a]	Day 20	
		Survival (% ± SD)	AFDW (mg/larva ± SD)
Negative control	<LOQ	83 ± 12	1.45 ± 0.16
Solvent control	<LOQ	88 ± 11	1.11 ± 0.11
13	11	92 ± 7	1.22 ± 0.09
25	25	83 ± 7	1.30 ± 0.33
50	52	67 ± 20	1.78 ± 0.66
100	92	75 ± 14	1.12 ± 0.30
200	180	6 ± 8*	0.76 ± 0.54[b]

[a] Mean-measured concentrations were determined (by the study author) from the Day 0 and Day 20 results.
[b] Excluded from statistical analysis due to significant effect on survival at this level.
* Statistically-significant compared to the negative control ($p<0.05$).

63-Day Endpoints:

Percent emergence averaged 77 and 60% for the negative and solvent control levels, respectively, compared to 71, 62, 55, 58, and 8% for the mean-measured 10, 21, 46, 79, and 140 µg ai/kg treatment levels, respectively. Differences were statistically-reduced compared to the negative control ($p<0.05$) at the 46 and 79 µg ai/kg levels. The 140-µg ai/kg level was excluded from statistical analysis due to a significant effect on Day-20 survival. It was reported that the statistical analysis should be interpreted with caution since the percent emergence data generated a weak dose-response, with all levels ≤79 µg ai/kg exceeding the control acceptability criteria (i.e., ≥50% emergence). Using mean-measured concentrations, the NOAEC and LOAEC for percent emergence were 21 and 46 µg ai/kg, respectively, and the 63-Day EC_{50} (with 95% C.I.) was 76 (67 to 87) µg ai/kg. Adjusted for the organic carbon content of the sediment (i.e., 1.9%), the NOAEC and LOAEC for percent emergence were 1.1 and 2.4 µg ai/g TOC, respectively, and the EC_{50} was 4.0 (3.5 to 4.6) µg ai/g TOC.

Nominal Sediment, µg ai/kg	Mean-measured Sediment, µg ai/kg	Day 63				
		Percent Emergence ± SD	Male Emergence Rate ± SD	Female Emergence Rate ± SD	Male Days to Death ± SD	Female Days to Death ± SD
Negative control	<LOQ	77 ± 11	0.0438 ± 0.0044	0.0381 ± 0.0029	3.7 ± 0.95	5.3 ± 1.0
Solvent control	<LOQ	60 ± 15	0.0388 ± 0.0049	0.0371 ± 0.0049	4.1 ± 1.0	4.7 ± 1.1
13	10	71 ± 24	0.0427 ± 0.0041	0.0356 ± 0.0049	3.7 ± 0.93	4.1 ± 0.79*
25	21	62 ± 13	0.0414 ± 0.0027	0.0379 ± 0.0029	4.3 ± 0.84	4.3 ± 0.88
50	46	55 ± 13*	0.0424 ± 0.0089	0.0400 ± 0.0067	3.1 ± 1.2	3.2 ± 1.2*
100	79	58 ± 13*	0.0400 ± 0.0055	0.0330 ± 0.0027	3.7 ± 1.1	4.1 ± 0.68
200	140	8 ± 8[(a)]	0.0288 ± 0.0108[(a)]	0.0276 ± 0.0061[(a)]	5 ± 0[(a)]	4.0 ± 0.82[(a)]

[(a)] Excluded from statistical analysis due to significant effect on Day-20 survival at this level.
* Statistically-significant compared to the negative control ($p<0.05$).

Statistical analysis of emergence rates for males and females determined no significant differences compared to the negative control up to and including the mean-measured 79 µg ai/kg level. At the negative control, solvent control, and mean-measured 10, 21, 46, 79, and 140 µg ai/kg treatment levels, male emergence rates averaged 0.0438, 0.0388, 0.0427, 0.0414, 0.0424, 0.0400, and 0.0288, respectively, and female emergence rates averaged 0.0381, 0.0371, 0.0356, 0.0379, 0.0400, 0.0330, and 0.0276, respectively. For both sexes, the NOAEC and LOAEC were 79 and >79 µg ai/kg, respectively, and the 63-Day EC_{50} was observed to be >140 µg ai/kg. When normalized for organic content of the sediment (i.e., 1.9%), the NOAEC and LOAEC were 4.2 and >4.2 µg ai/g TOC, and the EC_{50} was observed to be >7.4 µg ai/g TOC.

The time to death of male midges averaged 3.7 and 4.1 days for the negative and solvent control levels, respectively, and 3.7, 4.3, 3.1, 3.7, and 5 days for the mean-measured 10, 21, 46, 79, and 140 µg ai/kg treatment levels, respectively. No statistically-significant differences were indicated up to and including the 79 µg ai/kg level; the 140-µg ai/kg level was excluded from statistical analysis due to a significant effect on Day-20 survival. In females, the time to death averaged 5.3 and 4.7 days for the negative and solvent control levels, respectively, and 4.1, 4.3, 3.2, 4.1, and 4.0 days for the mean-measured 10, 21, 46, 79, and 140 µg ai/kg treatment levels, respectively. Differences were statistically-significant compared to the negative control ($p<0.05$) at the10 and 46 µg ai/kg levels; however, due to the lack of a clear dose response, the differences were considered to be incidental to exposure. Thus, for both sexes, the NOAEC and LOAEC were 79 and >79 µg ai/kg, respectively, and the 63-Day EC_{50} was observed to be >140 µg ai/kg. When normalized for organic content of the sediment (i.e., 1.9%), the NOAEC and LOAEC were

4.2 and >4.2 µg ai/g TOC, and the EC_{50} was observed to be >7.4 µg ai/g TOC.

Nominal Sediment, µg ai/kg	Mean-measured Sediment, µg ai/kg	Day 63				
		Egg Masses per Mated Female ± SD	Eggs per Egg Mass ± SD	Eggs per Mated Female ± SD	Percent Hatch ± SD	Days to Oviposition ± SD
Negative control	<LOQ	0.38 ± 0.25	798 ± 182	323 ± 251	97 ± 2	1.3 ± 0.39
Solvent control	<LOQ	0.23 ± 0.37	865 ± 120	205 ± 348	99 ± 0.3	1.3 ± 0.58
13	10	0.53 ± 0.30	665 ± 221	382 ± 272	97 ± 2	1.3 ± 0.38
25	21	0.51 ± 0.22	708 ± 231	359 ± 198	95 ± 5	1.4 ± 0.42
50	46	0.18 ± 0.19	867 ± 441	168 ± 227	95 ± 8	1.8 ± 1.5
100	79	0.76 ± 0.23	791 ± 142	590 ± 153	97 ± 2	1.2 ± 0.15
200	140	---[(a)]	---[(a)]	---[(a)]	---[(a)]	---[(a)]

[(a)] Excluded from statistical analysis due to significant effect on Day-20 survival at this level.

No treatment-related effects on reproduction were observed up to and including the mean-measured 79 µg ai/kg level. Specifically, there were no statistically-significant differences from the negative control indicated for the number of egg masses per mated female, the number of eggs per egg mass, the number of eggs per mated female, percent hatch, or the days to oviposition. Successful reproduction did not occur in survivors from the 140-µg ai/kg level. For the control through 79 µg ai/kg levels, the number of egg masses per mated female averaged 0.18 to 0.53, the number of eggs per egg mass averaged 665 to 867, the number of eggs produced per mated female averaged 168 to 590, percent hatch averaged 95 to 99%, and days to oviposition averaged 1.2 to 1.8. For all reproductive endpoints, the NOAEC and LOAEC were 79 and >79 µg ai/kg, respectively, and the EC_{50} was >79 µg ai/kg. When normalized for organic content of the sediment (i.e., 1.9%), the NOAEC and LOAEC were 4.2 and >4.2 µg ai/g TOC, and the EC_{50} was >4.2 µg ai/g TOC.

Analytical:

Concentrations of permethrin were determined in sediment and pore water on Days 0, 20, and 63. Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high K_{oc} values) and regular renewal of the overlying water. For the nominal 13, 25, 50, 100, and 200 µg ai/kg levels, sediment concentrations measured 11, 3, 43, 80, and 200 µg ai/kg, respectively, on Day 0; 10, 26, 61, 100, and 160 µg ai/kg, respectively, on Day 20; and 9.2, 15, 34, 54, and 72 µg ai/kg, respectively, on Day 63. Calculated mean-measured concentrations for Days 0 and 20 were 11, 25, 52, 92, and 180

µg ai/kg, respectively, and represented 83 to 100% of nominal sediment levels. Calculated mean-measured concentrations for the entire study were 10, 21, 46, 79, and 140 µg ai/kg, respectively, and represented 72 to 92% of nominal levels. For the nominal 13, 25, 50, 100, and 200 µg ai/kg levels, pore water concentrations measured 0.20, 0.26, 0.56, 0.76, and 1.7 µg ai/L, respectively, on Day 0; 0.17, 0.32, 0.57, 1.0, and 1.5 µg ai/L, respectively, on Day 20; and 0.80, 0.096, 0.19, 0.40, and 0.44 µg ai/L, respectively, on Day 63. Overall mean-measured pore water concentrations were 0.39, 0.22, 0.44, 0.73, and 1.2 µg ai/L, respectively.

B. Statistical Results (From Study Report)

Endpoints analyzed were survival and growth (ash-free dry weight; AFDW) on Day 20; and percent emergence, emergence rate (gender-specific), time to death (gender-specific), eggs masses per mated female, eggs per egg mass, eggs per mated female, percent hatch, and days to oviposition. Analyses were performed with CETIS™ (version 1.8.1.1, 2009) statistical software. Percent survival and percent emergence data were transformed (e.g., arcsine square-root percentage) prior to analysis. Results were provided in terms of mean-measured sediment concentrations (using 0 to 20 or 0 to 63 Days, as applicable), OC-normalized sediment concentrations, and estimated (freely-dissolved permethrin only) pore water concentrations (see Reviewer's Comments section).

A t-Test was used to compare the performance of the negative control and solvent control data. For all endpoints, data were statistically similar, and the treatment groups were compared to the negative control data to determine potential treatment-related effects.

For all endpoints, the data were tested for normality using the Shapiro-Wilks' Test, and for homogeneity of variance using a Modified Levene's Test or Bartlett's Test. Male emergence and time to oviposition data did not meet the assumption for normality, and time to oviposition also did not meet the assumption of homogeneity of variance. Thus, these endpoints were analyzed using the non-parametric Wilcoxon's Test with Bonferroni's Adjustment. All remaining endpoints met both assumptions and were assessed using Bonferroni's Adjusted t-Test. NOAEC and LOAEC values were assigned based on significance. All statistical analyses were conducted at the 95% level of certainty except in the case of the qualification tests (i.e., Shapiro-Wilks', Modified Levene's, and Bartlett's Tests), in which a 99% level of certainty was applied.

The LC/EC_{50} values with associated 95% confidence intervals were determined using the Trimmed Spearman-Karber method within CETIS™.

Endpoint	Methods	Mean-measured Sediment, µg ai/kg	OC-normalized Sediment, µg ai/g OC	Estimated Pore Water, ng ai/L
20-Day survival	Bonferroni's Adjusted t-Test	NOAEC: 92 LOAEC: 180 LC_{50}: 110 95% C.I.: 95 to 120	NOAEC: 4.8 LOAEC: 9.5 LC_{50}: 5.7 95% C.I.: 5.0 to 6.5	NOAEC: 17 LOAEC: 34 LC_{50}: 20 95% C.I.: 18 to 23
20-Day AFDW	Bonferroni's Adjusted t-Test	NOAEC: 92 LOAEC: >92 LC_{50}: >180 95% C.I.: N/A	NOAEC: 4.8 LOAEC: >4.8 LC_{50}: >9.5 95% C.I.: N/A	NOAEC: 17 LOAEC: >17 LC_{50}: >34 95% C.I.: N/A
Percent emergence	Bonferroni's Adjusted t-Test	NOAEC: 21 LOAEC: 46 LC_{50}: 76 95% C.I.: 67 to 87	NOAEC: 1.1 LOAEC: 2.4 LC_{50}: 4.0 95% C.I.: 3.5 to 4.6	NOAEC: 4.0 LOAEC: 8.7 LC_{50}: 15 95% C.I.: 13 to 17
Male emergence rate	Wilcoxon's Test with Bonferroni's Adjustment	NOAEC: 79 LOAEC: >79 LC_{50}: >140 95% C.I.: N/A	NOAEC: 4.2 LOAEC: >4.2 LC_{50}: >7.4 95% C.I.: N/A	NOAEC: 15 LOAEC: >15 LC_{50}: >27 95% C.I.: N/A
Female emergence rate	Bonferroni's Adjusted t-Test	NOAEC: 79 LOAEC: >79 LC_{50}: >140 95% C.I.: N/A	NOAEC: 4.2 LOAEC: >4.2 LC_{50}: >7.4 95% C.I.: N/A	NOAEC: 15 LOAEC: >15 LC_{50}: >27 95% C.I.: N/A

Endpoint	Methods	Mean-measured Sediment, µg ai/kg	OC-normalized Sediment, µg ai/g OC	Estimated Pore Water, ng ai/L
Male days to death	Bonferroni's Adjusted t-Test	NOAEC: 79 LOAEC: >79 LC_{50}: >140 95% C.I.: N/A	NOAEC: 4.2 LOAEC: >4.2 LC_{50}: >7.4 95% C.I.: N/A	NOAEC: 15 LOAEC: >15 LC_{50}: >27 95% C.I.: N/A
Female days to death	Bonferroni's Adjusted t-Test	NOAEC: 79 LOAEC: >79 LC_{50}: >140 95% C.I.: N/A	NOAEC: 4.2 LOAEC: >4.2 LC_{50}: >7.4 95% C.I.: N/A	NOAEC: 15 LOAEC: >15 LC_{50}: >27 95% C.I.: N/A
Egg masses/mated female	Bonferroni's Adjusted t-Test	NOAEC: 79 LOAEC: >79 LC_{50}: >140 95% C.I.: N/A	NOAEC: 4.2 LOAEC: >4.2 LC_{50}: >7.4 95% C.I.: N/A	NOAEC: 15 LOAEC: >15 LC_{50}: >27 95% C.I.: N/A
Eggs/egg mass	Bonferroni's Adjusted t-Test	NOAEC: 79 LOAEC: >79 LC_{50}: >140 95% C.I.: N/A	NOAEC: 4.2 LOAEC: >4.2 LC_{50}: >7.4 95% C.I.: N/A	NOAEC: 15 LOAEC: >15 LC_{50}: >27 95% C.I.: N/A
Eggs/mated female	Bonferroni's Adjusted t-Test	NOAEC: 79 LOAEC: >79 LC_{50}: >140 95% C.I.: N/A	NOAEC: 4.2 LOAEC: >4.2 LC_{50}: >7.4 95% C.I.: N/A	NOAEC: 15 LOAEC: >15 LC_{50}: >27 95% C.I.: N/A

Endpoint	Methods	Mean-measured Sediment, µg ai/kg	OC-normalized Sediment, µg ai/g OC	Estimated Pore Water, ng ai/L
Percent hatch	Bonferroni's Adjusted t-Test	NOAEC: 79 LOAEC: >79 LC_{50}: >140 95% C.I.: N/A	NOAEC: 4.2 LOAEC: >4.2 LC_{50}: >7.4 95% C.I.: N/A	NOAEC: 15 LOAEC: >15 LC_{50}: >27 95% C.I.: N/A
Days to oviposition	Wilcoxon's Test with Bonferroni's Adjustment	NOAEC: 79 LOAEC: >79 LC_{50}: >140 95% C.I.: N/A	NOAEC: 4.2 LOAEC: >4.2 LC_{50}: >7.4 95% C.I.: N/A	NOAEC: 15 LOAEC: >15 LC_{50}: >27 95% C.I.: N/A

Most Sensitive Endpoint(s): percent emergence

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method:

The reviewer statistically verified the results for Day 20 survival and ash-free dry weight (AFDW), time to death, time to oviposition, number of eggs per case and per female, and male/female and combined sex development rate using CETIS v. 1.8.7.12. with backend settings implemented by EFED on 03/25/14. The negative and solvent control groups were compared for all endpoints using an equal variance two-sample t-Test and subsequent comparisons to treatment were made using only the negative control. Male development was the only endpoint for which a significant control difference was detected ($p<0.05$). All endpoints were additionally tested to determine if data satisfied the assumptions of normality and homogeneity of variances. All endpoints satisfied parametric assumptions with the exception of male development rate number of eggs per primary egg case, and time to oviposition. The NOAEC/LOAEC values for these endpoints were determined using the Mann-Whitney Multiple Comparison Test, while the remaining NOAEC/LOAEC values (with the exception of percent emergence) were determined using Dunnett's test. Percent emergence was the only endpoint observed to follow a linearly decreasing monotonic trend, so the NOAEC/LOAEC values were determined using the Jonckheere-Terpstra test. The reviewer's analysis detected lower NOAEC and LOAEC values for percent emergence and eggs per mated female (number eggs per emerged female). This may be due to a difference in statistical methodology, as the study author used Bonferroni's Adjusted t-Test for most of the analyses.

The current CETIS test design for the 850.1760 chronic sediment chironomus study captures data for total number emerged and male/female number emerged, subsequently calculating percent emergence while the study author reported male and female emergence. The current CETIS design also reports total days to death, though the study author reported analysis results for male and female days to death. It also does not include reporting for percent hatched. The reviewer was unable to analyze male and female emergence, percent hatched, and days to death using the current test data setup.

14. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the most sensitive endpoint agreed with the study author's; however, the reviewer's analysis of this dose-dependent endpoint (using William's test) detected a lower NOAEC and LOAEC value than the study author.

The study author calculated LC_x and EC_x values for several endpoints. These were not verified by the reviewer. Guideline number 850.1760 in CETIS for Chronic Sediment *Chironomous* applies only to hypothesis testing and does not require that a regression analysis be conducted.

The reported toxicological values (i.e., NOAEC, LOAEC, LC/EC_{50}) for the Day-20 endpoints (survival and AFDW) were established using mean-measured sediment concentrations from the Day 0 and Day 20 intervals. Otherwise, toxicological values were established using mean-measured sediment concentrations from Days 0, 20, and 63. In addition to mean-measured sediment concentrations, results were reported (by the study author) in terms of OC-normalized sediment concentrations and estimated freely-dissolved levels of permethrin in pore water (as regular pore water analysis consisted of both the freely-dissolved fraction plus the fraction associated with the dissolved organic matter). The study author estimated the concentrations of freely-dissolved permethrin in the pore water using the following equation, and data are summarized in the following table.

$$C_{free} = (1000 \times C_{sed\ oc} / K_{oc})$$

where:
C_{free} = freely dissolved pore water concentration (ng ai/L)
$C_{sed\ oc}$ = OC-normalized concentration in sediment (µg ai/g OC)
K_{oc} = organic carbon partitioning coefficient

Nominal Sediment (µg ai/kg)	Mean-measured Sediment (µg ai/kg)	OC-Normalized Sediment (µg ai/g OC)	Freely-dissolved Pore Water (ng ai/L)	Mean-measured Pore Water (µg ai/L)
Days 0 and 20				
13	11	0.58	2.1	Not reported
25	25	1.3	4.7	Not reported
50	52	2.7	9.7	Not reported
100	92	4.8	17	Not reported
200	180	9.5	34	Not reported
Days 0, 20 and 63				
13	10	0.53	1.9	0.39
25	21	1.1	4.0	0.22
50	46	2.4	8.7	0.44
100	79	4.2	15	0.73
200	140	7.4	27	1.2

As concentrations of the test substance were variable in sediment at all except nominal 13 µg ai/kg level (lowest treatment level), TWA concentrations were reviewer-calculated (refer to copy of Excel worksheets in Section 16) for sediment and pore water. TWA calculations were very similar to mean-measured concentrations determined by the study author and were reported in the Executive Summary and Conclusions sections of the DER. TWA concentrations were

calculated by the reviewer using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
C TWA is the time-weighted average concentration,
C j is the concentration measured at time interval j (j = 0, 1, 2,...n)
t j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j (e.g., t 0 = 0 hours (test initiation), t 1 =24 hours, t 2 =96 hours).

Dosing stock solutions and treated sediment from all levels (prior to allocation into the replicate vessels) were analyzed for permethrin. Recoveries in the stock solutions ranged from 100 to 110% of nominal concentrations. Analysis of the spiked sediment following dosing and prior to allocation into the replicate exposure vessels ranged from 84 to 110% of nominal concentrations.

The analytical method used to quantify permethrin in formulated sediment was validated on May 24, 2010. Fortified samples were extracted three times with methanol:purified reagent water and hexane (1:1, v:v); the organic extracts were combined and purified for analysis using solid phase extraction (SPE). Aliquots were analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization mode (GC/MS/NCI). The method validation established an average recovery of 103 ± 5.73% (CV = 5.57%) from artificial sediment fortified at 0.00100 and 2.00 µg ai/kg. The limit of quantitation (LOQ) was 0.000215 µg ai/kg.

The method to quantify the amount of permethrin in freshwater was validated in February 2011. Freshwater was fortified with the test substance at nominal concentrations of 0.0100 (sample LOQ), 0.0300, 0.200, and 0.500 µg ai/L. Fortified samples were acidified and extracted twice with ethyl acetate. The extracts were evaporated to dryness, and the residues re-constituted in 0.1% peanut oil in acetone, and aliquots were analyzed using GC/MS/NCI. Recoveries of permethrin averaged 84.9 ± 12.3% (CV = 14.5%). It was reported that due to the low concentrations tested, the LOQ was set at 0.0100 µg ai/L; sample LOQ recoveries averaged 103 ± 8.02% (CV=7.78%).

Chromatograms of permethrin consist of two peaks due to the two isomers present. It was reported that for the validations and most of the analyses, the permethrin concentration was determined using the sum of the areas of both peaks. However, for some of the analyses, there was an interfering peak present overlapping one of the isomer peaks. As a result, it was reported that these samples were quantified using the area from only one of the isomer's peaks, and that as QC sample concentrations met the acceptance criteria and were determined in the same manner, that this deviation had minimal impact on the test results.

Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high K_{oc} values) and regular renewal of the overlying water.

The dissolved organic carbon (DOC), total organic carbon (TOC), ammonia (as N), temperature, and pH were measured in isolated pore water at each level on Days 0, 20, and 63. The DOC decreased from 160 to 210 mg/L on Day 0 to 66 to 87 mg/L by Day 63. Similarly, the TOC decreased from 170 to 220 mg/L on Day 0 to 67 to 110 mg/L by Day 63. Ammonia levels ranged from 4.2 to 4.7 mg/L on Day 0, 1.4 to 2.0 mg/L on Day 20, and ≤0.10 to 1.1 mg/L on Day 63. Temperature measured 21°C throughout the study, and the pH ranged from 6.3 to 6.9 throughout the study.

Definitive study dates were May 24 to Judy 26, 2011.

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16. COPY OF REVIEWER'S TWA CONCENTRATION CALCULATIONS:

SEDIMENT Nominal Concentration (ug ai/kg)	Time (Day)	SEDIMENT Measured Concentration (ug ai/kg)	SEDIMENT TWA (ug ai/kg)
13.0	0	11	
	20	10	
	63	9.2	
			10
25	0	23	
	20	26	
	63	15	
			22
50	0	43	
	20	61	
	63	34	
			49
100	0	80	
	20	100	
	63	54	
			81
200	0	200	
	20	160	
	63	73	
			137

SEDIMENT Nominal Concentration (ug ai/kg)	Time (Day)	PORE WATER Measured Concentration (ug ai/L)	PORE WATER TWA (ug ai/L)
13.0	0	0.2	
	20	0.17	
	63	0.8	
			0.39
25	0	0.26	
	20	0.32	
	63	0.096	
			0.23
50	0	0.56	

	20	0.57	
	63	0.19	
			0.44
100	0	0.76	
	20	1	
	63	0.4	
			0.76
200	0	1.7	
	20	1.5	
	63	0.44	
			1.2